Journal of Steroid Biochemistry & Molecular Biology 113 (2009) 189–194

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

Testosterone action on erythropoiesis does not require its aromatization to estrogen: Insights from the testosterone and estrogen treatment of two aromatase-deficient men☆

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ARTICLE INFO

Article history:
Received 21 February 2008
Received in revised form
27 November 2008
Accepted 22 December 2008

Keywords:
Hemoglobin
Hematocrit
Estrogen deficiency
Estradiol treatment
Testosterone treatment
Sex steroids
Erythropoiesis
Man

ABSTRACT

Androgens act on erythropoiesis, but the relative role of testosterone (T) and estradiol (E_2) on erythropoietic parameters in men is a poorly investigated issue. In order to evaluate separately the effects on erythropoiesis of high-dose T administration alone and of physiological dose of E_2 administration alone two adult men with aromatase deficiency were assessed before and during each treatment. Blood cell count, hemoglobin (Hb), hematocrit (Hct), erythrocyte mean cell volume (MCV), erythrocyte mean corpuscular hemoglobin (MCH), erythrocyte mean corpuscular hemoglobin concentration (MCHC), serum ferritin, iron and total iron-binding capacity (TIBC), serum erythropoietin, serum total testosterone and estradiol were evaluated. Hb, Hct and red cell count rose during testosterone treatment, consistently with the increase in circulating testosterone, but failed to increase during estradiol treatment. A decrease in Hb, Hct and red cell count was recorded in one of the two subjects during estradiol treatment, with a concomitant decrease in serum testosterone. Circulating T alone is capable of and sufficient to influence erythropoiesis, especially at supraphysiological dosage, while circulating E_2 have not the same effect on erythropoietic parameters, suggesting the hypothesis that the erythropoietic changes induced by androgens are not mediated via its aromatization to estrogens.

1. Introduction

The effects of androgens on erythropoiesis have been well understood since 1940s [1,2] and androgens were the main pharmacologic agents used for erythropoietic stimulation before the availability of recombinant hematopoietic growth factors [3]. On the other hand, estrogens action on erythropoiesis has not received the same attention, leaving a lack of knowledge about the respective role of each sex steroid on the erythropoietic parameters.

Androgen action on erythropoiesis [4] is highlighted by the gender differences in blood hemoglobin [5–7] and by the changes in circulating hemoglobin that occur only in boys at puberty [8,9]. Consistently, hemoglobin concentration (Hb) and hematocrit (Hct) are higher in men than in women [5–7], with this gender difference absent before puberty. In particular, pubertal increase of Hb occurs only in boys at puberty, with an approx five-month delay with respect to the increase in circulating testosterone levels [8,9]. This gender differences in erythropoiesis have been traditionally ascribed to the amount of androgens that is greater in men than in women, but they could be also related to menstrual blood loss in women [10], even though evidence on this latter mechanism are controversial [11]. Furthermore, the androgen deprivation in men reduces erythropoiesis [12–16] and hypogonadism is frequently associated with reduced Hct [17]. Both conditions are reversible with androgen replacement treatment [18] or by removing androgen deprivation [16,19]. Finally, age-related anemia is at least partially due to the decline in circulating androgens levels in older men [20] and polycythemia represents an undesired risk for testosterone replacement treatment that needs monitoring [21]. All these findings suggest that sex hormones regulate erythropoiesis, that androgens may trigger pubertal up-regulation of erythropoiesis in boys [22] and that they may play a role in the maintenance of Hb levels typical of adult males [4,18].

Although the mechanisms by which androgens exert their influence on erythropoiesis have not been completely clarified yet, it seems that testosterone may act directly on bone marrow stem cells [3,4,16,23–25] rather than indirectly by increasing erythropoietin

☆ Grant: This work was supported by grant from Ministero dell'Università e della Ricerca (MUR, ex-40%-2005).

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doi:10.1016/j.jsbmb.2008.12.007

(Epo) [26], the latter being a potent and effective agent for red cells synthesis stimulation [27,28].

The possibility that androgens may act on erythrocytosis via their conversion to estrogens, as it happens in other biological actions in men [29,30], is still a poorly investigated issue. Nevertheless, gender differences in erythropoiesis at puberty [8,9,22] and during adulthood [4,7], and the erythropoietic effects of non-aromatizable androgens [31], all indirectly argue against this hypothesis. Even several pathophysiological models suggest that androgens directly modulate erythropoiesis and that their conversion to estrogens does not play a role in this biological function. Consistently, androgen excess in women leads to a red cell count higher than normal, such as in females with congenital adrenal hyperplasia [32] or those receiving large doses of androgens [33]. On the other hand, a possible effect of estrogen on erythropoiesis has been postulated on the basis of the results of several studies. Recently, high doses of estrogen administered to male-to-female transsexuals resulted in a decrease of both Hct and Hb concentrations, but the concomitant administration of an antiandrogen drug did not permit to establish if the reduction of erythropoiesis was due to the direct action of estrogen or if it was related to androgen deficiency or both [34]. The most important evidence that rose the hypothesis of a possible role of estrogens in male erythropoiesis comes from a recent study showing a direct association between hematocrit and serum estradiol in men treated with testosterone [35]. A direct action of estrogen on erythropoiesis was previously shown also in women [36] as well as in animal studies [37]. However, all these studies suffer either from the presence of an intact aromatase enzyme, meaning that exogenous androgens may still be converted into estrogens in women, or from an intact hypothalamic feedback, causing exogenous estrogens to lead to a concomitant but not complete androgen suppression in men. Thus, the relative effect of each sex steroid on erythropoiesis remains an as yet unsolved issue in men. Thus, to check if androgens may act on erythropoiesis via their conversion to estrogen is of importance on a physiological viewpoint, especially if recent data on this issue are considered [34,35].

The aim of this study was to evaluate separately the effects of supraphysiological testosterone treatment and those of physiological estradiol treatment on some parameters of erythropoiesis in two adult men with aromatase deficiency. This investigation model is complementary to the study protocols in which estrogen suppression obtained by using aromatase inhibitors has been limited by the potency of the drugs administered [22,38]. Indeed, human congenital complete aromatase deficiency is a more useful model for testing separately the androgen and estrogens effects [30,39,40], since in these patients the conversion pathway of androgen to estrogen is not functioning.

2. Materials and methods

The study was carried out on two male subjects affected by aromatase deficiency; their detailed clinical and genetic studies have already been published [39,40]. The subject here referred to as Subject 1 is the aromatase-deficient man described by Carani et al. in 1997 [39,41], while Subject 2 is the aromatase-deficient man described by Maffei et al. in 2004 [40], who was also affected by concomitant mild hypogonadism [40,41]. Both subjects were treated with supraphysiological doses of testosterone at the beginning of their clinical management, and thereafter, once estrogen deficiency was diagnosed, transdermal estradiol treatment was started.

The effects of a supraphysiological dose of testosterone administration alone [testosterone enanthate 250 mg i.m. every 10 days (Subject 1) and every 15 days (Subject 2) lasting at least 6 months), and a physiological dose of estradiol administration alone (transdermal estradiol 25 μg twice weekly for 6 months) on erythropoiesis were evaluated before and during each treatment. Both testosterone treatment and estrogen treatment lasted a minimum of six months, and the period of washout between the two treatments was longer than twelve months. Testosterone treatment was performed until the diagnosis of aromatase deficiency had been made in order to attain bone maturation [39,40]. The study design is summarized in detail in Fig. 1 and in particular it is illustrated the timing of biochemical evaluation before and after at least six months of each treatment (Fig. 1).

2.1. Biochemical analyses

The routine laboratory investigations included a complete blood count (erythrocyte, leukocyte and platelet count) hemoglobin, hematocrit, erythrocyte mean cell volume (MCV), erythrocyte mean corpuscular hemoglobin (MCH), erythrocyte mean corpuscular hemoglobin concentration (MCHC), serum ferritin, iron and total iron-binding capacity (TIBC). The following parameters were also subsequently evaluated on stored samples: serum Epo, serum total testosterone and serum estradiol. All these parameters were evaluated during each phase of the study. The study was performed, for Subject 1, at the Endocrine Unit of the University of Modena and Reggio Emilia, and for, Subject 2, at the Consultorios Asociados de Endocrinologia of Buenos Aires, Argentina. Serum samples of both men were stored separately, but serum measurements of Epo, testosterone and estradiol performed on stored samples were subsequently all assayed at the Endocrine Unit of the University of Modena and Reggio Emilia. Blood count and the other hematological parameters except Epo were performed at the time of blood collection and the data were obtained from the patient's record charts.

Blood samples for the analysis of both blood and serum were collected after an overnight fast, at 08:00 h, by venipuncture of the antecubital vein. Serum samples obtained by centrifugation were stored at −80 °C, until assayed. Blood sample was collected at the second week of the 6th month of therapy for both treatments and after 7 days from the administration of testosterone enanthate during testosterone treatment.

Hematologic parameters (Hb, Hct, MCV, MCH, MCHC, and blood cell count) were analyzed using an automated cell counter [Cell-Dyn 3500 (Abbott Laboratories, Abbott Park, IL, USA)] [42,43].

Serum ferritin, iron and TIBC were measured by commercially available kits.

Serum Epo was quantified using an immunochemiluminometric assay on an IMMULITE analyzer (DPC, Los Angeles, Calif). The detection limit of the assay is 0.2 U/L. The within-run coefficient of variation (CV) was <9% and the total CV <9% in the concentration ranged from 7 to 148 U/L.

Serum total testosterone was measured using commercial RIA (Diagnostic product corp., Los Angeles, CA). The inter-assay and the intra-assay coefficients of variation for testosterone were 11% and 5% respectively. The cross-reactivity with 5α-dihydrotestosterone was 2.8%; antibody cross-reactivity against less potent androgens (androstenedione, 3β-androstanediol, dehidroepiandrosterone) and other possible interfering steroids was less than 1%.

Serum estradiol levels were determined using a commercially available double antibody RIA (Third-Generation DSL-39100, Diagnostic Systems Laboratories, Inc., Webster, TX) with a sensitivity of 0.6 pg/mL (2.2 pmol/L), the lowest standard at 1.5 pg/mL (5.5 pmol/L), linearity to 150 pg/mL (550 pmol/L), and an ED50 of 20 pg/mL (73 pmol/L). The cross-reactivity with estrone and with less potent estrogens was less than 7 and 0.45%, respectively. The inter-assay and the intra-assay coefficients of variation for estradiol were 4.1–9.9 and 3.4–3.9%, respectively.

Both subjects gave written and informed consent for both treatments and for the publication of the data.